Technical Data Sheet

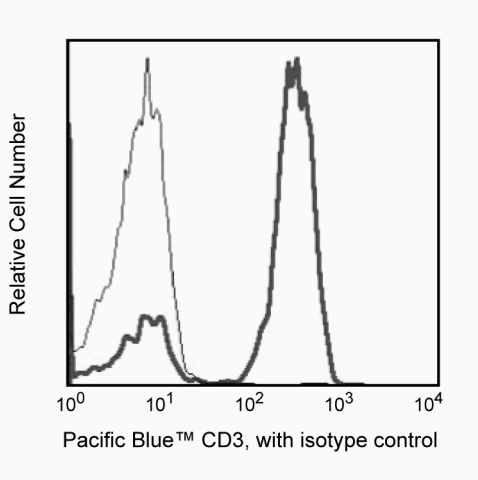
Pacific Blue™ Mouse Anti-Human CD3

Product Information

Material Number:	**558117**
Alternate Name:	CD3e; CD3E; T3E; TCRE; T-cell surface antigen T3/Leu-4 epsilon
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	UCHT1
Immunogen:	Human infant thymocytes and peripheral blood lymphocytes from a Sézary Syndrome donor
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human
Workshop:	III 471
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium azide.

Description

The UCHT1 monoclonal antibody specifically binds to the human CD3ε-chain, a 20-kDa subunit of the CD3/T cell antigen receptor complex. CD3ε is expressed on 70-80% of normal human peripheral blood lymphocytes and 60-85% of thymocytes. Studies from the HLDA Workshop show that this antibody is mitogenic for CD3ε-positive cells when used in conjunction with costimulatory agents such as pokeweed mitogen or anti-CD28 antibody. CD3 plays a central role in signal transduction during antigen recognition. The UCHT1 antibody stains both surface and intracellular CD3ε unlike the other CD3 clone, HIT3a, that stains only extracellular CD3ε.



Flow cytometric analysis of CD3 expression on human peripheral blood lymphocytes. *Whole blood was stained with either Pacific Blue™ Mouse IgG1, κ Isotype Control (Cat. No. 558120; dashed line histogram) or Pacific Blue™ Mouse Anti-Human CD3 (Cat. No. 558117; solid line histogram). Erythrocytes were lysed with Lysing Buffer (Cat. No. 555899). Fluorescence histograms were derived from events with the forward and side light-scattering characteristics of viable lymphocytes.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody is conjugated to Pacific Blue™ under optimum conditions, and unreacted Pacific Blue™ was removed.

Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
558120	Pacific Blue™ Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
349202	BD FACS™ Lysing Solution	100 mL	(none)
555899	Lysing Buffer	100 mL	(none)

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For country contact information, visit **bdbiosciences.com/contact**

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
6. Pacific Blue™ has a maximum absorption of 416 nm and maximum emission of 451 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
7. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Barclay NA, Brown MH, Birkeland ML, et al, ed. *The Leukocyte Antigen FactsBook.* San Diego, CA: Academic Press; 1997(Biology)

Beverley PC, Callard RE. Distinctive functional characteristics of human "T" lymphocytes defined by E rosetting or a monoclonal anti-T cell antibody. *Eur J Immunol.* 1981; 11(4):329-334. (Biology)

Knapp W. W. Knapp .. et al., ed. *Leucocyte typing IV : white cell differentiation antigens.* Oxford New York: Oxford University Press; 1989:1-1182. (Clone-specific)

Lanier LL, Allison JP, Phillips JH. Correlation of cell surface antigen expression on human thymocytes by multi-color flow cytometric analysis: implications for differentiation. *J Immunol.* 1986; 137(8):2501-2507. (Biology)

McMichael AJ. A.J. McMichael .. et al., ed. *Leucocyte typing III : white cell differentiation antigens.* Oxford New York: Oxford University Press; 1987:1-1050. (Clone-specific)

Schlossman SF. Stuart F. Schlossman .. et al., ed. *Leucocyte typing V : white cell differentiation antigens : proceedings of the fifth international workshop and conference held in Boston, USA, 3-7 November, 1993.* Oxford: Oxford University Press; 1995(Biology)

Schlossman SF. Stuart F. Schlossman .. et al., ed. *Leucocyte typing V : white cell differentiation antigens : proceedings of the fifth international workshop and conference held in Boston, USA, 3-7 November, 1993.* Oxford: Oxford University Press; 1995(Clone-specific)

Monoclonal Antibodies Detecting Human Antigens

CD10 (HI10a)

Form	Catalog number
FITC	340925
PE	340921
PE-Cy7	341092
APC	340923
APC-H7	655404
APC-R700	659120

Product availability varies by region. Contact BD Biosciences Customer Support or your local sales representative for information.

RESEARCH APPLICATIONS

Research applications include:

- Characterization of non-T (common) acute lymphoblastic leukemias[1,2]
- Analysis of early stages of hematopoietic differentiation[3–5]
- Analysis of neutrophil chemotaxis[6–8]

DESCRIPTION

Specificity

The CD10 antibody recognizes a 100-kilodalton (kDa) type II transmembrane, glycosylated, zinc-containing metalloprotease.[9,10] The CD10 antigen is also known as common acute lymphoblastic leukemia antigen (CALLA), neutral endopeptidase (NEP), gp100, and enkephalinase.[11]

Antigen distribution

The CD10 antigen is found on lymphocytes from samples with acute B-lymphoid leukemia.[12] The CD10 antigen is also present on a wide variety of normal and neoplastic cell types including renal epithelium, fibroblasts, granulocytes, germinal center B lymphocytes,[13] neutrophils,[6,7,14] some T-cell leukemias,[15] and some lymphoma, melanoma, and glioma cell lines.[11]

The CD10 antigen cleaves a number of biologically active peptides,[16] including fMLP, and may modulate the chemotactic activity of fMLP towards neutrophils.[8] Inhibition of the CD10 antigen promotes B-cell maturation,[17] suggesting that it plays a role in B-cell development.

Clone

The CD10 antibody, clone HI10a,[10] is derived from the hybridization of P3-63-Ag8.653 mouse myeloma cells with spleen cells isolated from BALB/c mice immunized with blasts from a patient with acute CALLA leukemia.

Composition

The CD10 antibody is composed of mouse IgG_1 heavy chains and kappa light chains.

Product configuration

The following are supplied in phosphate buffered saline (PBS) containing a stabilizer and a preservative.

Form	Number of tests	Volume per test (µL)[a]	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
FITC	50	20	12.5	1	12.5	Gelatin	0.1% Sodium azide
PE	50	20	6	1	6	Gelatin	0.1% Sodium azide
PE-Cy™7	100	5	12.5	0.5	25	Gelatin	0.1% Sodium azide
APC	100	5	12.5	0.5	25	Gelatin	0.1% Sodium azide

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Form	Number of tests	Volume per test (µL)[a]	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
APC-H7	100	5	25	0.5	50	BSA	ProClin® 300
APC-R700[b]	100	5	6.25	0.5	12.5	BSA	ProClin 300

a. Volume required to stain 10^6 cells.
b. BD Horizon™ APC-R700

CAUTION Some PE-Cy7, APC-H7, and APC-R700 conjugates show changes in their emission spectra with prolonged exposure to paraformaldehyde or light. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

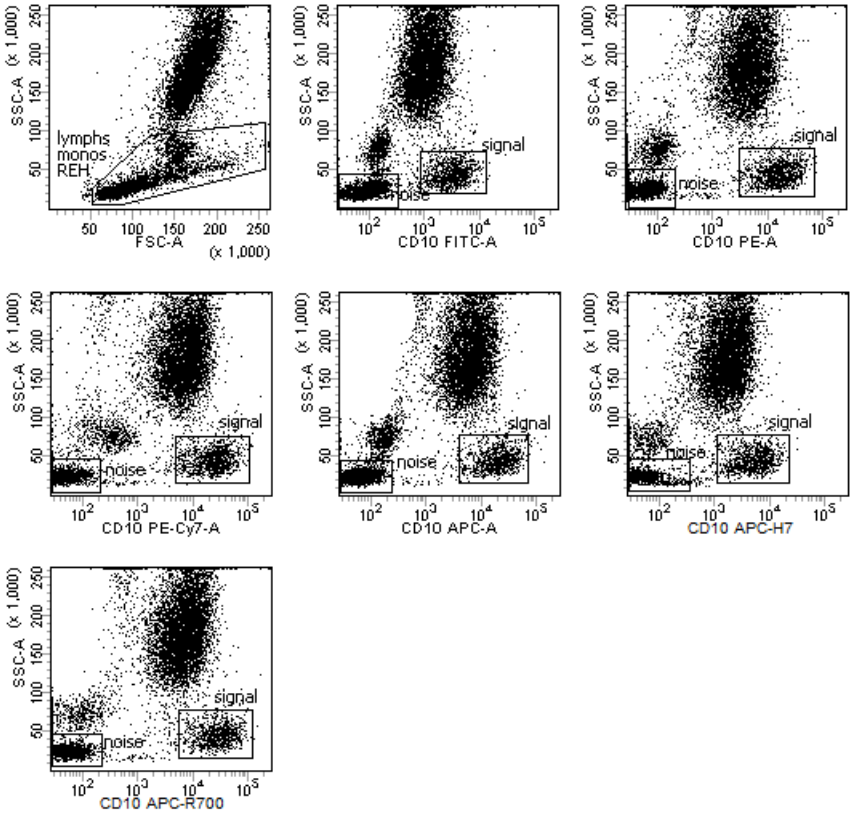
PROCEDURE

Visit our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

REPRESENTATIVE DATA

Flow cytometric analysis was performed on 10^6 REH cells added per mL of whole blood stained with the indicated conjugated antibody and gated on lymphocytes, monocytes, and REH cells. Laser excitation was at 488 nm, 635 nm, or 640 nm.

The APC-R700 conjugate is read off the red laser (640 nm) using a 685 longpass mirror with a 712/21 bandpass filter. Representative data analyzed with a BD FACS™ brand flow cytometer is shown in the following plots.



HANDLING AND STORAGE

Store vials at 2°C–8°C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

WARNING

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[18,19] and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing and gloves.

Some reagents are bottled with ProClin 300, and contain 0.003% of a mixture of CMIT/MIT (3:1), CAS number 55965-84-9.

Warning

H317 May cause an allergic skin reaction.

Wear protective gloves/eye protection. Wear protective clothing. Avoid breathing mist/vapours/spray. If skin irritation or rash occurs: Get medical advice/attention. IF ON SKIN: Wash with plenty of water. Dispose of contents/container in accordance with local/regional/national/international regulations.

CHARACTERIZATION

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

WARRANTY

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

THE PRODUCTS SOLD HEREUNDER ARE WARRANTED ONLY TO CONFORM TO THE QUANTITY AND CONTENTS STATED ON THE LABEL OR IN THE PRODUCT LABELING AT THE TIME OF DELIVERY TO THE CUSTOMER. BD DISCLAIMS HEREBY ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY AND FITNESS FOR ANY PARTICULAR PURPOSE AND NONINFRINGEMENT. BD'S SOLE LIABILITY IS LIMITED TO EITHER REPLACEMENT OF THE PRODUCTS OR REFUND OF THE PURCHASE PRICE. BD IS NOT LIABLE FOR PROPERTY DAMAGE OR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING PERSONAL INJURY, OR ECONOMIC LOSS, CAUSED BY THE PRODUCT.

REFERENCES

1. Hann IM, Richards SM, Eden OB, Hill FGH. Analysis of the immunophenotype of children treated on the Medical Research Council United Kingdom Acute Lymphoblastic Leukaemia Trial XI (MRC UKALLXI). *Leukemia*. 1998;12:1249-1255.
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8. Hofman P, Selva E, Le Negrate G, et al. CD10 inhibitors increase f-Met-Leu-Phe-induced neutrophil transmigration. *J Leukoc Biol*. 1998;63:312-320.
9. Greaves MF. Monoclonal antibodies as probes for leukemic heterogeneity and hematopoietic differentiation. In: Knapp W, ed. *Leukemia Markers*. New York, NY: Academic Press; 1981:19.
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18. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline — Third Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2005. CLSI document M29-A3.

19. Centers for Disease Control. Perspectives in disease prevention and health promotion update: universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens in health-care settings. *MMWR*. 1988;37:377-388.

PATENTS AND TRADEMARKS

BD CD43 (1G10)

Monoclonal Antibodies Detecting Human Antigens

Form	Catalog number
APC-H7	655407

Product availability varies by region. Contact BD Biosciences Customer Support or your local sales representative for information.

Research Applications

Research applications include:

- Research on T cell-macrophage interactions[1]
- Research on neutrophil spreading and movement[2,3]
- Study of *Streptococcus gordonii* DL1 binding to myeloid cells[4]

Description

Specificity

The CD43 antibody reacts specifically with the major 95–135-kilodalton (kDa) sialoglycoprotein found on most human leucocytes.[5] The CD43 antigen is also known as leukosialin or sialophorin.

Antigen distribution

The CD43 antigen is expressed on T cells, natural killer (NK) cells, pre-B and activated B cells,[5] granulocytes,[6] and neutrophils.[3] The CD43 antigen is differentially expressed on subpopulations of mononuclear phagocytic cells.[7] It is not present on most peripheral blood resting B cells, erythrocytes, and non-hematopoietic cells.[5] Additionally, expression of the CD43 antigen by committed myeloid, erythroid, and lymphoid progenitors has been reported.

The CD43 antigen may be involved in the regulation of B, T, and NK cell function.[5,8–10] The CD43 antigen plays a role in cell adhesion, which can be positive or negative, depending on the context.[2,11] The CD43 antigen serves as a counter-receptor for CD169, the prototypical member of the sialic acid binding Ig-like lectins (Siglec) family expressed on macrophages.[1]

Clone

The CD43 antibody, clone 1G10,[5] is derived from the hybridization of X63 mouse myeloma cells with spleen cells isolated from mice immunized with a lymph node suspension from a patient with Hodgkin lymphoma.

Composition

The CD43 antibody is composed of mouse IgG_1 heavy chains and kappa light chains.

Product configuration

The following is supplied in buffer containing a stabilizer and a preservative.

Form	Number of tests	Volume per test (µL)	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
APC-H7	100	5	25	0.5	50	BSA	CMIT/MIT (3:1)

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

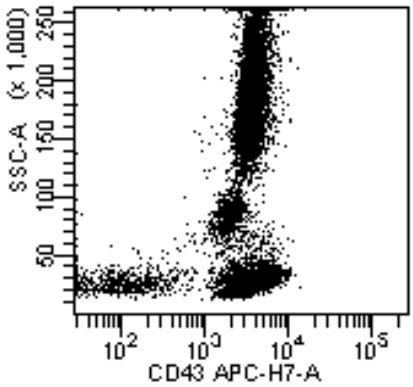
CAUTION Some APC-H7 conjugates show changes in their emission spectra with prolonged exposure to paraformaldehyde or light. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

Procedure

Go to our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

Representative Data

Flow cytometric analysis was performed on whole blood stained with the indicated conjugated antibody. Laser excitation was at 635 nm. Representative data analyzed with a BD flow cytometer is shown in the following plot.



Handling and Storage

Store vials at 2–8 °C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

Warning

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[12,13] and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

The APC-H7 conjugate contains a mixture of 0.00236% 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H -isothiazol-3-one [CMIT/MIT (3:1)], CAS number 55965-84-9. These reagents are classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

	Warning
	H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects.
Prevention	P261: Avoid breathing dust/fume/gas/mist/vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves/protective clothing/eye protection/face protection. P273: Avoid release to the environment.

	Warning
Response	P302+P352: IF ON SKIN: Wash with plenty of water. P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P362+P364: Take off contaminated clothing and wash it before reuse.
Disposal	P501: Dispose of contents/container to an approved facility in accordance with local, regional, national and international regulations.

Go to regdocs.bd.com to download the Safety Data Sheet.

Characterization

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

Warranty

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

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References

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Patents and Trademarks

For US patents that may apply, see bd.com/patents.

Contact Information

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BD OneFlow™ Setup Beads

25 tests per kit—Catalog No. 658620

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1. INTENDED USE

BD OneFlow™ Setup beads are intended to set voltages appropriate for the BD multicolor tube assay when used with a suitably equipped BD™ flow cytometer and software designated for in vitro diagnostic use.

2. SUMMARY AND EXPLANATION

BD OneFlow Setup beads are fluorescent particles that are used to set cytometer detector photomultiplier tube voltages (PMTVs) for the BD multicolor tube assay. PMTVs are manually adjusted to place the BD OneFlow Setup beads at their lot specific median fluorescence intensity (MFI) target ranges for all fluorescence parameters. Lysed washed blood (LWB) is used to set cytometer FSC and SSC voltages to a target value range. The detector settings are then saved as Application Settings.

3. PRINCIPLES OF THE PROCEDURE

BD has developed a suite of beads that are used with BD FACSDiva™ software to standardize setup of the BD FACSCanto™ II flow cytometer with a 3-laser, 8-color 4-2H-2V BD default (4-2H-2V) optical configuration. First, BD FACSDiva™ CS&T IVD beads (CS&T IVD beads) are used to perform daily cytometer quality control. BD OneFlow Setup beads and LWB are then used to set assay-specific PMTVs and to generate Application Settings. Finally, BD™ FC beads 8-color kit for BD OneFlow™ assays (BD FC beads) is used to calculate compensation.

4. STORAGE AND HANDLING

- Store the vial at 2°C–8°C. The vial should not be frozen. Protect from exposure to light. The beads are stable until the expiration date shown on the vial label when stored as directed. Do not use after the expiration date. Do not mix the contents of one kit with another. Target values can vary between lots and this could result in inaccurate detector settings.
- After dilution, the beads are stable for
  - 1 hour at 18°C–25°C
  - 8 hours at 2°C–8°C

  WARNING Protect the diluted bead suspension from light. Some of the dyes used to manufacture the beads are very light sensitive. Fluorescence levels can change if the beads are exposed to direct light for longer than 20 minutes.

5. REAGENTS AND MATERIALS

Reagents provided

- One vial of BD OneFlow Setup beads, sufficient for 25 tests

  BD OneFlow Setup beads are supplied in phosphate buffered saline (PBS) with bovine serum albumin (BSA) and 0.1% sodium azide.
- Monthly MFI target range card

  The monthly MFI target range card contains MFI ranges for all fluorescence detectors.
- Daily MFI target range card

  The daily MFI target range card contains MFI ranges for all fluorescence detectors that are optimized for optional daily monitoring. See the *Instrument Setup Guide for BD OneFlow Assays* for more information.

Reagents and materials required but not provided

- Installer CD with OneFlow Setup template (Catalog No. 659305)

  The template contains two global worksheets (*BD OneFlow™ TMFI Setup* and *BD OneFlow™ Scatter Setup*). Be sure to order this CD prior to using the BD OneFlow Setup beads for the first time.
- Vortex mixer
- Pasteur pipets
- Micropipettor with tips
- 12 x 75-mm capped polystyrene tubes
- BD FACS Flow™ sheath fluid (Catalog No. 342003)
- BD FACSCanto II flow cytometer with a 4-2H-2V optical configuration

  See the cytometer user's guide for information.
- BD FACSDiva software v8.0.1 or later

  See the *BD FACSDiva Software Reference Manual*.
- BD FACSDiva CS&T IVD beads (Catalog No. 656046 or 656047)

  See the *BD FACSDiva CS&T IVD Beads* IFU.
- Lysed washed blood (LWB) specimen from a normal donor

  Use the blood specimen within 24 hours of collection. See Lysing the blood specimen for instructions.

- BD FACS™ lysing solution (Catalog No. 349202)

  For dilution instructions and warnings, see the reagent IFU.

- Wash buffer (filtered PBS with 0.5% BSA and 0.09% sodium azide)

Precautions

- For in vitro diagnostic use.
- Do not use BD OneFlow Setup beads beyond their expiration date or beyond the day-of-use stability period after dilution, as described in the Storage and Handling section. Beads used beyond their stability period begin to lose fluorescence, which may result in inaccurate PMTV setup.
- MFI target ranges provided on the monthly MFI target range card are bead lot specific. Verify that the bead lot number on the monthly MFI target range card matches the lot ID of the BD OneFlow Setup beads that you are using. A mismatch will result in inaccurate PMTVs and Application Settings.

6. PROCEDURE

Generate new Application Settings using BD OneFlow Setup beads and LWB at the following times:

- Once a month to ensure consistent and accurate assay-specific PMTV setup
- Each time a new lot of BD OneFlow Setup beads is used
- Each time a new lot of CS&T IVD beads is used
- Whenever a new baseline is defined using CS&T IVD beads
- After cytometer maintenance or service is performed

Installing the OneFlow Setup template

1. Insert the installer CD into the CD drive and click the installer icon.
2. Follow the prompts to install the template.

   The installer will copy and paste the template into the folder: D:\BDExport\Templates\Panel\BDPanels.

Lysing the blood specimen

You will use a LWB specimen to adjust FSC and SSC voltages.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[1,2] and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

1. Add 100 µL of whole blood from a normal donor to a tube labeled *LWB*.
2. Add 2 mL of 1X BD FACS lysing solution.
3. Vortex 3–5 seconds to mix well.
4. Incubate for 10 minutes at 18°C–25°C.
5. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.
6. Remove the supernatant without disturbing the cell pellet and leave approximately 50 µL of residual liquid in the tube.
7. Vortex 3–5 seconds to resuspend the cell pellet.

8. Add 2 mL of wash buffer to the tube.
9. Vortex 3–5 seconds to mix well.
10. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.
11. Remove the supernatant without disturbing the cell pellet and leave approximately 50 µL of residual liquid in the tube.
12. Vortex 3–5 seconds to resuspend the cell pellet.
13. Add 250 µL of wash buffer to the tube.
14. Vortex 3–5 seconds to mix well.
15. Save the LWB sample to adjust FSC and SSC voltages. See Adjusting FSC and SSC on page 6.

    Store at 2°C–25°C until acquisition.

Preparing BD OneFlow Setup beads

Before preparing BD OneFlow Setup beads, verify that the CS&T IVD beads daily performance check for the 4-2H-2V configuration was completed today and passed.

1. Label a 12 x 75-mm capped polystyrene tube *Setup beads*.
2. Thoroughly mix the BD OneFlow Setup beads vial.
3. Prepare the diluted beads according to Table 1 and the task you are performing.

Table 1 BD OneFlow Setup beads preparation

Task	BD FACSFlow sheath fluid (µL)	Beads (number of drops)
First time setup	700	2
Monthly setup	350	1

4. Return the BD OneFlow Setup beads to 2°C–8°C storage.
5. Vortex the tube gently before use.

   If not acquiring immediately, store the diluted beads, protected from light, for up to:

   - 1 hour at 18°C–25°C
   - 8 hours at 2°C–8°C

Setting up the software

1. In the BD FACSDiva workspace title bar, confirm that the 4-2H-2V optical configuration is selected.
2. From the menu bar, select **Experiment > New Experiment > Blank Experiment**, then click **OK**.
3. If prompted by the CST Mismatch dialog, select **Use CST Settings**.
4. Rename the experiment with the run date appended with OneFlow (for example, OneFlow Setup_today's date).
5. From the menu bar, select **Experiment > New Specimen**.

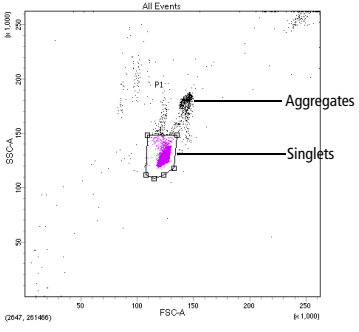
   The **Panel Template** window opens.
6. Click the **BD Panels** tab and select the **OneFlow Setup** template, then click OK.
7. Click **Cytometer Settings** in the Browser window.
8. In the Inspector, select the **Parameters** tab and ensure that **FSC-A**, **FSC-H**, **SSC-A**, and **SSC-H** are all selected.
9. Navigate to the **Compensation** tab in the Inspector and deselect the **Enable Compensation** option.

Adjusting PMTVs

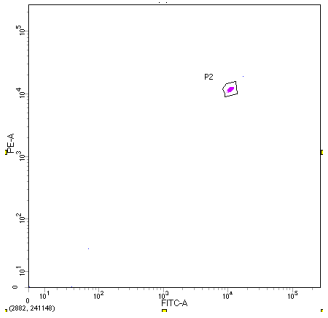
1. In the **Browser**, set the current tube pointer to the BD OneFlow Setup beads tube.
2. In the Acquisition Dashboard, set **Events To Record** to 5,000.
3. Vortex the beads tube.
4. Install the tube on the cytometer.
5. Adjust the flow rate to **Low**, and click **Acquire Data**.

   NOTE It may take 10–15 seconds until events begin to appear.
6. In the FSC-A vs SSC-A dot plot, adjust the **P1** gate to include only the singlet bead population (no aggregates).

   NOTE Click the **Increase** button in the **Tools** menu of the global worksheet to see more detail in the FSC-A vs SSC-A dot plot.



7. In the FITC-A vs PE-A dot plot, adjust the **P2** gate to include only the singlet bead population.



8. In the Cytometer window, select the **Parameters** tab and adjust the voltages for FITC, PE, PerCP-Cy5.5, PE-Cy7, APC, APC-H7, V450, and V500 so that the MFI of the bead population in the P2 gate falls within the corresponding range on the monthly MFI target range card (Figure 1).

Figure 1 Example monthly MFI target range card

BD OneFlow™ Setup Beads (Monthly)

REF LOT

Fluorophore	Min (-2%)	MFI	Max (+2%)
FITC	10397	10610	10822
PE	11896	12139	12382
PERCP-CY5.5	46584	47535	48486
PE-CY7	22194	22647	23100
APC	57164	58331	59497
APC-H7	129387	132028	134668
V450	9639	9835	10032
V500-C	24076	24568	25059

Monthly Target Ranges 23-16178-00

9. If needed, increase the size of the **P2** gate to ensure that the singlet bead population remains within the gate while adjusting the PMTVs.

Experiment Name:	OneFlow Setup_20140627	CYTOMETER CONFIG CRE...	2007-01-02T12:00:00-08:00
Specimen Name:	PMT Setup	CST PERFORMANCE EXPI...	2014-06-24T11:57:03-07:00
Tube Name:	OneFlow Setup Bead_001	CST REGULATORY STAT...	CE-IVD Performance Check
Record Date:	Jun 23, 2014 3:13:44 PM	CST BEADS EXPIRED:	False
CYTOMETER CONFIG NA...	3-laser, 8-color (4-2H-2V) (B...		

Population	FITC-A Median	PE-A Median	PerCP-Cy5-5-A Median	PE-Cy7-A Median
P2	10,654	12,196	47,223	22,513

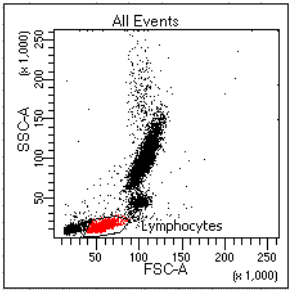
Population	APC-A Median	APC-H7-A Median	V450-A Median	V500-A Median
P2	58,579	132,245	9,751	24,481

10. Click **Record Data**.
11. Verify that the MFI values fall within range.

Adjusting FSC and SSC

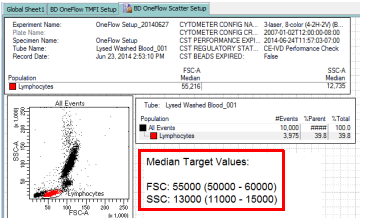
NOTE Use the normal LWB sample that you prepared for this procedure.

1. In the **Browser**, select the current tube pointer for the LWB tube.
2. In the Acquisition Dashboard, confirm that the **Events To Record** are set to 10,000 total events.
3. Vortex the LWB tube.
4. Install the LWB sample on the cytometer and confirm that the flow rate is set to **Low**.
5. Click **Acquire Data**.
6. In the Cytometer window, select the **Parameters** tab and lower the voltages for FSC and SSC so that the lymphocyte population is on scale.
7. In the Cytometer window, select the **Threshold** tab and set the FSC threshold to 10,000.



8. Adjust the Lymphocyte gate to encompass the entire lymphocyte population in the FSC vs SSC dot plot.
9. Adjust the FSC and SSC voltages to place the lymphocyte population within the FSC-A and SSC-A target value ranges given on the BD OneFlow Scatter Setup worksheet. See Figure 2.

Figure 2 Statistics view on worksheet



10. If needed, re-adjust the lymphocyte gate.
11. Click **Record Data**.
12. Verify that the MFI values fall within range.

13. Right-click **Cytometer Settings > Application Settings > Save**, and click **OK**.

    CAUTION Use the default name for the Application Settings. Do not rename the Application Settings.

14. When prompted, click **Yes** to maintain the modified threshold values.

7. LIMITATIONS

- BD OneFlow Setup beads are intended to set voltages appropriate for the BD multicolor tube assay when used with a BD FACSCanto II flow cytometer set with the 4-2H-2V optical configuration and BD FACSDiva software v8.0.1 or later.
- The PMT voltages and Application Settings generated using the BD OneFlow Setup beads are intended to be used for the BD multicolor tube assay and should not be used for any other clinical reagents or assays.
- BD OneFlow Setup beads do not perform as a fluorescence calibrator and should not be used for setting up a flow cytometer for quantitative fluorescence measurements.

8. PERFORMANCE CHARACTERISTICS

Performance of the BD OneFlow Setup beads was established by testing at BD Biosciences laboratories in San Jose, CA.

Accuracy

Accuracy testing was performed using BD FACSDiva software v8.0.1 or later on BD FACSCanto II flow cytometers using BD OneFlow Setup beads (test method), Sphero™* Rainbow calibration particles (reference method), and BD FC beads (used as stable fluorescent particles). On each cytometer, detector gain settings were generated using BD OneFlow Setup beads and Sphero Rainbow calibration particles by placing the beads within the bead lot-specific target MFI ranges specified for each detector. BD FC beads were acquired using each gain setup generated with the test and reference methods. Average MFI of the positive BD FC beads were compared between the test and reference methods. Data is shown in Table 2.

Table 2 Accuracy of MFI values between test and reference methods (relative mean bias)

Channel	% Relative bias	SD[a]
FITC	–0.30	1.16
PE	–0.30	1.43
PerCP-Cy5.5	0.46	2.93
PE-Cy7	1.70	2.25
APC	2.49	3.06
APC-H7	2.25	3.28
V450	–4.41	5.04
V500	0.27	0.85

a. SD= Standard deviation

Precision

Precision testing was performed using BD FACSDiva software v8.0.1 or later on multiple BD FACSCanto II flow cytometers using multiple lots of BD OneFlow Setup beads over multiple days. BD FC beads were used as stable fluorescent particles. Detector gain settings were generated using BD OneFlow Setup beads by placing the beads within the bead lot-specific target

* Sphero is a trademark of Spherotech, Inc.

MFI ranges specified for each detector. Using the PMT gain settings generated for each setup, the eight single color BD FC beads were acquired. Percent CV of the MFI values of the positive BD FC beads were used to verify precision. Data is shown in Table 3.

Table 3 BD OneFlow Setup beads precision (lot to lot and instrument to instrument)

Channel	%CV[a]	UCL[b]
FITC	8.6	10.2
PE	3.4	4.0
PerCP-Cy5.5	17.0	20.2
PE-Cy7	3.9	4.7
APC	1.3	1.5
APC-H7	2.8	3.3
V450	17.1	20.3
V500	4.8	5.7

a. CV = Coefficient of variation
b. UCL = Upper confidence limit of the 95% confidence interval

WARRANTY

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THE PRODUCTS SOLD HEREUNDER ARE WARRANTED ONLY TO CONFORM TO THE QUANTITY AND CONTENTS STATED ON THE LABEL OR IN THE PRODUCT LABELING AT THE TIME OF DELIVERY TO THE CUSTOMER. BD DISCLAIMS HEREBY ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY AND FITNESS FOR ANY PARTICULAR PURPOSE AND NONINFRINGEMENT. BD'S SOLE LIABILITY IS LIMITED TO EITHER REPLACEMENT OF THE PRODUCTS OR REFUND OF THE PURCHASE PRICE. BD IS NOT LIABLE FOR PROPERTY DAMAGE OR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING PERSONAL INJURY, OR ECONOMIC LOSS, CAUSED BY THE PRODUCT.

TROUBLESHOOTING

Problem	Possible Cause	Solution
No beads detected	Beads not mixed prior to diluting	Vortex the beads vial, prepare a fresh suspension of beads according to Table 1, and re-run the tube.
	Beads too dilute	
	Debris in the beads suspension	
	Incorrect beads used	
	Air bubbles in the flow cell or sheath filter	Check the fluidics for bubbles and debris. See the cytometer IFU for more information.
	Clogs within the sample tubes and lines	Check the fluidics for clogs and debris. See the cytometer IFU for more information.
	Back pressure in the waste lines	Check the waste tank vent for obstructions. See the cytometer IFU for more information.
	High scatter noise (FSC or SSC)	Perform monthly maintenance. See the cytometer IFU for more information. Call BD Biosciences.
	FSC threshold is set too high	Lower the FSC threshold.
	FSC and SSC PMTVs are not optimum	Optimize FSC and SSC PMTVs.

Problem	Possible Cause	Solution
No cells detected in lysed, washed blood sample	Air bubbles in the flow cell or sheath filter	Check the fluidics for bubbles and debris. See the cytometer IFU for more information.
	Clogs within the sample tubes and lines	Check the fluidics for clogs and debris. See the cytometer IFU for more information.
	Back pressure in the waste lines	Check the waste tank vent for obstructions. See the cytometer IFU for more information.
	Cell concentration in prepared samples is too low	Prepare a new sample.
	FSC and SSC PMTVs not optimum for cells	Optimize FSC and SSC PMTVs.

REFERENCES

1. *Protection of Laboratory Workers from Occupationally Acquired Infections—Third Edition; Approved Guideline*. Wayne, PA: Clinical and Laboratory Standards Institute; 2005. CLSI document M29 A-3.
2. Centers for Disease Control. Perspectives in disease prevention and health promotion update: universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens in health-care settings. *MMWR*. 1988:37:377-388.

BD FACS™ Lysing Solution

Catalog No. 349202

23-1358(14)
2023-04
English

℞ Only CE IVD

1. INTENDED USE

BD FACS™ Lysing Solution is intended for lysing red blood cells for flow cytometric applications. It can be used in both lyse/wash and lyse/no-wash procedures.

2. SUMMARY OF THE TEST

Efficient detection of leukocytes in specimens depends on the elimination of interfering cells. Whole blood lysis has been shown to be as effective as density gradient centrifugation in the preparation of peripheral blood mononuclear cells (PBMCs) for lymphocyte subset analysis.[1,2,3,4] In clinical laboratories, whole blood lysis methods have essentially replaced Ficoll-Paque™ density gradient separation because of shorter sample preparation time and less handling of whole blood.[5] Studies have also shown that the lysed whole blood method is less likely to show loss of leukocyte subsets and may help improve assay reproducibility when compared to earlier methods.[5,6,7]

BD FACS™ Lysing Solution is intended for use by laboratory professionals.

Principle of Operation

When the specimen is added to the antibody reagent, the fluorochrome-labeled antibodies in the reagent bind specifically to leukocyte surface antigens. The stained samples are then treated with BD FACS™ Lysing Solution, which lyses red blood cells (RBCs) under gentle hypotonic conditions while preserving the leukocytes.

3. REAGENT

Reagent Composition

BD FACS™ Lysing Solution is a proprietary buffered solution containing formaldehyde and diethylene glycol.

Precautions

BD FACS™ Lysing Solution contains 31.34% ethanol, 2,2´-oxybis- (diethylene glycol) (CAS number 111-46-6, EC number 203-872-2), 9.77% formaldehyde (CAS number 50-00-0, EC number 200-001-8), and 3.43% methanol (CAS number 67-56-1, EC number 200-659-6). The lysing solution is classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Regulation (EC) No 1272/2008, and 29 CFR 1910.1200. Go to regdocs.bd.com/regdocs/sdsSearch to download the Safety Data Sheet.

	Danger
	H302+H312+H332: Harmful if swallowed, in contact with skin or if inhaled. H314: Causes severe skin burns and eye damage. H317: May cause an allergic skin reaction. H335: May cause respiratory irritation. H341: Suspected of causing genetic defects. H350: May cause cancer. H370: Causes damage to organs. H373: May cause damage to organs through prolonged or repeated exposure. US only: H402: Harmful to aquatic life.
Prevention	P201: Obtain special instructions before use. P202: Do not handle until all safety precautions have been read and understood. P260: Do not breathe dust/fume/gas/mist/vapors/spray. P264: Wash face, hands and any exposed skin thoroughly after handling. P270: Do not eat, drink or smoke when using this product. P271: Use only outdoors or in a well-ventilated area. P272: Contaminated work clothing should not be allowed out of the workplace. P273: Avoid release to the environment. P280: Wear protective gloves/protective clothing/eye protection/face protection.
Response	P301+P330+P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. P312: Call a POISON CENTER or doctor/physician if you feel unwell. P303+P361+P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower]. P363: Wash contaminated clothing before reuse. P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P304+P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing. P310: Immediately call a POISON CENTER/doctor. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P307+P311: IF exposed: Call a POISON CENTER or doctor/ physician. P308+P313: If exposed or concerned: Get medical advice/attention.
Storage	P405: Store locked up.
Disposal	P501: Dispose of contents/container to an approved facility in accordance with local, regional, national and international regulations.

Storage and Handling

- BD FACS™ Lysing Solution (10X) is stable until the expiration date shown on the bottle label when stored as directed.
- The storage temperature is 2–25 °C.
- Do not use this reagent if discoloration occurs or a precipitate forms.

4. INSTRUMENT

BD FACS™ Lysing Solution is designed for flow cytometers equipped with appropriate computer hardware and software. The flow cytometer must be equipped to detect forward scatter (FSC) and side scatter (SSC).

5. SPECIMEN COLLECTION AND PREPARATION

See the instructions for use (IFU) for the reagent you are using for information about specimens supported.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[8,9] and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

6. PROCEDURE

Reagents and Materials

Reagents and materials provided

BD FACS™ Lysing Solution is provided as 100 mL of a 10X concentrate. After dilution, this volume is sufficient for 2,000 tests when used in lyse/no-wash procedures or for 500 tests when used in lyse/wash procedures.

Reagents and materials required but not provided

- 1X BD FACS™ Lysing Solution, diluted as described
- BD fluorochrome-conjugated antibodies to human leukocyte antigens
- Vortex mixer
- Micropipettor with tips
- Other materials might be required. Refer to the appropriate reagent IFU for more information.

Diluting BD FACS™ Lysing Solution

Dilute the 10X concentrate 1:10 with room temperature (20–25 °C) deionized water. The prepared solution is stable for 1 month when stored in a glass or high density polyethylene (HDPE) container at room temperature.

Staining the Specimen

Stain the specimen following instructions in the appropriate reagent IFU. Lyse RBCs as directed using diluted (1X) BD FACS™ Lysing Solution.

7. LIMITATIONS

- Samples with nucleated erythrocytes show incomplete lysis of RBCs because BD FACS™ Lysing Solution does not lyse nucleated erythrocytes as efficiently as enucleated RBCs. This may also occur when assaying blood samples from patients with certain hematologic disorders in which RBCs are difficult to lyse, as in myelofibrosis, sickle-cell anemia, thalassemia, and spherocytosis.[7,8]
- BD FACS™ Lysing Solution was developed for use with BD flow cytometers.
- BD FACS™ Lysing Solution was developed using EDTA as the anticoagulant. BD has limited information concerning use of other anticoagulants such as heparin.

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1. de Paoli P, Reitano M, Battistin S, Castiglia C, Santini G. Enumeration of human lymphocyte subsets by monoclonal antibodies and flow cytometry: a comparative study using whole blood or mononuclear cells separated by density gradient centrifugation. *J Immunol Methods*. 1984;72:349-353.
2. Ashmore LM, Shopp GM, Edwards BS. Lymphocyte subset analysis by flow cytometry. Comparison of three different staining techniques and effects of blood storage. *J Immunol Methods*. 1989;118:209-215.
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5. Jackson A. Basic phenotyping of lymphocytes: selection and testing of reagents and interpretation of data. *Clin Immunol Newslett.* 1990;10:43-55.
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8. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document M29-A4.
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NOTICE

EU Only: Users shall report any serious incident related to the device to the Manufacturer and National Competent Authority.

Outside EU: Contact your local BD representative for any incident or inquiry related to this device.

WARRANTY

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PATENTS AND TRADEMARKS

For US patents that may apply, see bd.com/patents.

HISTORY

Revision	Date	Changes made
23-1358(13)	2021-11	Updated to meet requirements of Regulation (EU) 2017/746.
23-1358(14)	2023-04	Updated legal manufacturer address. Added EU and Swiss importer addresses and importer symbol. Updated symbols glossary. Added Rx only symbol.

Symbols Glossary

Please refer to product labeling for applicable symbols.

Symbol	Meaning
	Manufacturer
EC REP	Authorized representative in the European Community
CH REP	Authorised representative in Switzerland
	Date of manufacture
	Use-by date
LOT	Batch code
REF	Catalogue number
SN	Serial number
STERILE	Sterile
STERILE A	Sterilized using aseptic processing techniques
STERILE EO	Sterilized using ethylene oxide
STERILE R	Sterilized using irradiation
STERILE	Sterilized using steam or dry heat
	Do not resterilize
	Non-sterile
	Do not use if package is damaged and consult *instructions for use*
STERILE	Sterile fluid path
STERILE EO	Sterile fluid path (ethylene oxide)
STERILE R	Sterile fluid path (irradiation)
	Fragile, handle with care
	Keep away from sunlight
	Keep dry
	Lower limit of temperature
	Upper limit of temperature
	Temperature limit
	Humidity limitation
	Biological risks
	Do not re-use
	Consult *instructions for use* or consult electronic *instructions for use*
	Caution
LATEX	Contains or presence of natural rubber latex
IVD	In vitro diagnostic medical device
CONTROL -	Negative control
CONTROL +	Positive control
	Contains sufficient for <*n*> tests
	For IVD performance evaluation only
	Non-pyrogenic
#	Patient number
	This way up
	Do not stack

Symbol	Meaning
	Single sterile barrier system
PHT DEHP BBP	Contains or presence of phthalate: combination of bis(2-ethylhexyl) phthalate (DEHP) and benzyl butyl phthalate (BBP)
	Collect separately Indicates separate collection for waste of electrical and electronic equipment required.
CE	CE marking; Signifies European technical conformity
	Device for near-patient testing
	Device for self-testing
Rx Only	This only applies to US: "Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner."
CC	Country of manufacture "CC" shall be replaced by either the two letter or the three letter country code.
	Collection time
	Cut
	Peel here
12	Collection date
	Keep away from light
H_2	Hydrogen gas is generated
	Perforation
	Start panel sequence number
	End panel sequence number
	Internal sequence number
/	<Box #> / <Total Boxes>
MD	Medical device
	Contains hazardous substances
	Ukrainian conformity mark
FC	Meets FCC requirements per 21 CFR Part 15
c UL us	UL product certification for US and Canada
UDI	Unique device identifier
	Importer
	Place patient label in framed area only
MR	Magnetic resonance (MR) safe
MR	Magnetic resonance (MR) conditional
MR	Magnetic resonance (MR) unsafe
For use with	For use with
This Product Contains Dry Natural Rubber	This Product Contains Dry Natural Rubber
For Export Only	For Export Only
Instruments	Instruments

Note: Text layout in symbols is determined by label design.

L006715(08) 2023-03

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